

two meniscus cannot meet each other. Without the meniscus touching each other, fluid would not be drawn into the second through hole. Both fluid transfer and fluid interface diffusion occur by contacting the two substrates filled with fluids in the through holes.

Paragraph [0033],

The present invention allows fluid transfer to the top substrate called fluid drawing, fluid transfer to the bottom substrate called fluid delivery and fluid interface diffusion called diffusional mixing. Therefore, this is an integrated platform for high throughput screening where a complicated and expensive dispenser is not needed. A large number of fluid array can be used where parallel fluid transfer and/or mixing occurs across the entire wafer. This allows building of a large library of fluids for the various purposes of combinatorial assay development, assay implementation, combinatorial chemical library synthesis, fluid storage and an environment for cell culturing. The "~~lip~~ collar" on the substrate can also make the top substrate self align with the bottom substrate. This makes positioning the top substrate very easy. In addition, the top substrate can be covered by a thin plastic layer such as a tape, to prevent evaporation of the fluid. The bottom through hole opening of the bottom substrate is much smaller compared to the top side that the evaporation would be minimized.

#### **In the Claims:**

1. (currently amended) A microfluidic system comprising:
  - a first substrate having at least one microfluidic through-hole between first and second surfaces of the first substrate, the through-hole having a cross-section sufficient to retain a liquid therein; and
  - a first substrate having at least one peak protruding from the second surface of the first substrate, the at least one

through-hole of the first substrate being in fluid communication with the tip of the at least one peak.

a second substrate having at least one microfluidic through-hole in a predetermined proximate spaced position and alignment with the at least one microfluidic through-hole of the first substrate, the at least one through-hole of the second substrate operable to retain the liquid displaced from the at least one through-hole of the first substrate thereto.

2. (original) The microfluidic system, as set forth in claim 1, further comprising means for displacing a liquid retained in the at least one through-hole of the first substrate to the at least one through-hole of the second substrate.

3. (original) The microfluidic system, as set forth in claim 1, further comprising means for aligning the at least one microfluidic through-hole of the first and second substrates.

4. (original) The microfluidic system, as set forth in claim 1, further comprising a physically driven force generated by meniscus contact between the liquids for displacing a liquid retained in the at least one through -hole of the first substrate to the at least one through-hole of the second substrate.

5. (original) The microfluidic system, as set forth in claim 1, further comprising a chemically driven force generated by meniscus contact between the liquids for displacing a liquid retained in the at least one through -hole of the first substrate to the at least one through-hole of the second substrate.

6. (original) The microfluidic system, as set forth in claim 1, further comprising a physically driven force generated by a pressure differential along the thickness of the substrates for

displacing a liquid retained in the at least one through -hole of the first substrate to the at least one through -hole of the second substrate.

7. (original) The microfluidic system, as set forth in claim 1, further comprising a physically driven force generated by an electrokinetic differential along the thickness of the substrates for displacing a liquid retained in the at least one through -hole of the first substrate to the at least one through -hole of the second substrate.

8. (original) The microfluidic system, as set forth in claim 1, further comprising a spacer disposed between the first and second substrates and operable to maintain an air gap in the predetermined spaced position between the first and second substrates.

9. (original) The microfluidic system, as set forth in claim 1, wherein the first and second substrates each further comprises hydrophobic upper and lower surfaces.

10. (original) The microfluidic system, as set forth in claim 1, wherein the at least one microfluidic through -hole of the second substrate comprises a tapered inner wall.

11. (original) The microfluidic system, as set forth in claim 1, wherein the at least one microfluidic through -hole of the first substrate comprises a tapered inner wall.

12. (original) The microfluidic system, as set forth in claim 1, wherein the at least one microfluidic through -holes of the first and second substrates each comprises a tapered inner wall.

13. (cancelled)

14. (currently amended) The microfluidic system, as set forth in claim ~~13~~ 1, wherein the at least one peak of the first substrate is operable to be at least partially accommodated within the at least one microfluidic through-hole of the second substrate to make contact with the liquid retained therein.

15. (original) The microfluidic system, as set forth in claim 1, wherein the first substrate further comprises a collar surrounding the opening of the at least one microfluidic through-hole in the second surface.

16. (cancelled)

17. (cancelled)

18. (cancelled)

19. (original) The microfluidic system, as set forth in claim 1, wherein the first and second substrates comprise a hydrophobic coating on the first and second surfaces thereof except for a predetermined distance from each through-hole opening.

20. (original) The microfluidic system, as set forth in claim 19, wherein the first and second substrates further comprise a non-hydrophobic surface between the hydrophobic coating and the through-hole openings.

21. (original) The microfluidic system, as set forth in claim 1, further comprising a plurality of microfluidic through-holes in each of the first and second substrates.

22. (currently amended) A microfluidic system comprising:  
a first substrate having an array of microfluidic through-holes having a first predetermined geometry between first and

second surfaces of the first substrate, the through-holes operable to retain a liquid therein by physical and/or chemical fluidic forces;

a first substrate having at least one peak protruding from the second surface of the first substrate, the at least one through-hole of the first substrate being in fluid communication with the tip of the at least one peak.

a second substrate having an array of microfluidic through-holes having a second predetermined geometry and in a predetermined proximate spaced position and alignment with the microfluidic through-holes of the first substrate, the through-holes of the second substrate operable to receive and retain a liquid retained and then displaced from the through-holes of the first substrate to the through-holes of the second substrate, the through-holes operable to retain the liquid by physical and/or chemical fluidic forces; and

an applied force operable to displace the liquid retained in the through-holes of the first substrate to the through-holes of the second substrate.

23. (original) The microfluidic system, as set forth in claim 22, further comprising means for aligning the microfluidic through-holes of the first and second substrates.

24. (original) The microfluidic system, as set forth in claim 22, wherein applied force comprises a force generated by meniscus contact between the liquids in the through-holes in the first and second substrates.

25. (original) The microfluidic system, as set forth in claim 22, wherein the applied force comprises a physically driven force generated by a pressure differential along the thickness of the substrates.

26. (original) The microfluidic system, as set forth in claim 22, wherein the applied force comprises a physically driven force generated by an electrokinetic differential along the thickness of the substrates.

27. (original) The microfluidic system, as set forth in claim 22, further comprising a spacer disposed between the first and second substrates and operable to maintain an air gap in the predetermined spaced position between the first and second substrates.

28. (original) The microfluidic system, as set forth in claim 22, wherein the first and second substrates each further comprises hydrophobic upper and lower surfaces.

29. (original) The microfluidic system, as set forth in claim 22, wherein the first and second geometries of the through-holes are the same.

30. (original) The microfluidic system, as set forth in claim 22, wherein the first and second geometries of the through-holes are different.

31. (original) The microfluidic system, as set forth in claim 22, wherein the second predetermined geometries of the microfluidic through-holes of the second substrate comprises a tapered inner wall.

32. (original) The microfluidic system, as set forth in claim 22, wherein the first and second predetermined geometries of the microfluidic through-holes of the first and second substrates comprise a tapered inner wall.

33. (original) The microfluidic system, as set forth in claim 22, wherein the first and second predetermined geometries of the

microfluidic through -holes of the first and second substrates comprise a conically tapered inner wall.

34. (original) The microfluidic system, as set forth in claim 22, wherein the first and second predetermined geometries of the microfluidic through -holes of the first and second substrates comprise a pyramiddally tapered inner wall.

35. (original) The microfluidic system, as set forth in claim 22, wherein the microfluidic through -holes of the first substrate each reaching the second surface at a peak protruding therefrom.

36. (original) The microfluidic system, as set forth in claim 33, wherein each peak of the first substrate is operable to be at least partially accommodated within the microfluidic through -hole of the second substrate to make contact with the liquid retained therein.

37. (original) The microfluidic system, as set forth in claim 22, wherein the first substrate further comprises a collar surrounding the opening of each microfluidic through -hole and protruding beyond the second surface.

38. (cancelled)

39. (cancelled)

40. (original) The microfluidic system, as set forth in claim 22, wherein the first and second substrates comprise a hydrophobic coating on the first and second surfaces thereof except for a predetermined distance from each through-hole opening.

41. (cancelled)

42. (original) The microfluidic system, as set forth in claim 22, further comprising a third substrate disposed adjacent the second substrate and having a plurality of microwells disposed in a surface of the substrate facing the second substrate, the microwells operable to receive the liquid displaced from the microfluidic through-holes of the first substrate to the microfluidic through-holes of the second substrate and then to the microwells.

43. (original) The microfluidic system, as set forth in claim 42, further comprising a channel defined between the second and third substrates operable to guide the liquid from the microfluidic through-holes of the second substrate to the microwells in the third substrate.

44. (original) The microfluidic system, as set forth in claim 22, wherein the through-holes in the first substrate are in in-line alignment with the through-holes in the second substrate.

45. (original) The microfluidic system, as set forth in claim 22, wherein the through-holes in the first substrate are in offset alignment with the through-holes in the second substrate.

46. (original) The microfluidic system, as set forth in claim 45, further comprising a channel defined in the predetermined space between the first and second substrates and in fluid communication with selected through-holes of the first and second substrates.

47. (original) A method of transferring liquids between first and second substrates, comprising:



loading a first liquid into a plurality of microfluidic through-holes disposed in the first substrate, the first liquid being retained in the through-holes;

loading a second liquid into a plurality of microfluidic through-holes disposed in the second substrate, the second liquid being retained in the through-holes; and

transferring the first liquid in the first substrate into the through-holes of the second substrate induced by meniscus contact between the first and second liquids and an applied force.

48. (original) The method, as set forth in claim 47, wherein transferring the first liquid comprises creating an electrical field differential across the first and second substrates.

49. (original) The method, as set forth in claim 47, wherein transferring the first liquid comprises creating a pneumatic differential across the first and second substrates.

50. (original) The method, as set forth in claim 47, further comprising transferring the liquid in the through-holes of the second substrate to microwells disposed in a third substrate.

51. (original) The method, as set forth in claim 47, further comprising positioning the first and second substrates so that the through-holes therein are in alignment with one another.

52. (original) The method, as set forth in claim 47, further comprising positioning the first and second substrates so that a fluid-conducting channel is formed between the first and second substrates, the channel being in fluid communication with at least selected ones of through-holes in the first and second substrates.

53. (original) The method, as set forth in claim 47, wherein loading the through -holes of the first and second substrates comprises immersing the first and second substrates into the respective first and second liquids.

54. (original) The method, as set forth in claim 53, wherein loading the through -holes of the first and second substrates comprises:

immersing the first and second substrates into respective containers containing the respective first and second liquids; and

evacuating gases from the respective containers.

55. (original) A method comprising:

loading a first test sample into a plurality of microfluidic through-holes disposed in the first substrate, the first test samples being retained in the through-holes;

loading a reagent into a plurality of microfluidic through-holes disposed in the second substrate, the reagent being retained in the through-holes; and

transferring the reagent in the second substrate into the through-holes of the first substrate induced by an applied force;

positioning and aligning a third substrate having a plurality of microfluidic through -holes with the microfluidic through-holes of the first substrate and forming a fluid -conducting channel in fluid communication with the through -holes of the first and third substrates between the first and third substrates; and

flushing the test sample reagent mixture in the through holes of the first substrate with a washing liquid introduced into the through-holes of the third substrate.

56. (original) The method, as set forth in claim 55, wherein transferring the reagent comprises creating an electrical field differential across the first and second substrates.

57. (original) The method, as set forth in claim 55, wherein transferring the reagent comprises creating a pneumatic differential across the first and second substrates.

58. (original) The method, as set forth in claim 55, further comprising transferring the test sample and reagent in the through-holes of the first substrate to microwells disposed in a fourth substrate.

59. (original) A method of preparing samples comprising:  
introducing an array of samples into a first substrate retained thereby by various fluid imbalance forces;  
positioning a second substrate adjacent the first substrate and receiving an array of spots of samples therefrom onto the second substrate;  
positioning a third substrate adjacent the second to further create another array of samples on the second substrate;  
repeating the above step with additional substrates to build a library of samples on the second substrate; and  
positioning and aligning the second substrate with the library of samples adjacent to an assay reagent substrate having an array of assay reagents.

60. (original) The method, as set forth in claim 59, further comprising transferring the samples to the second substrate by